*pnemoniae*[7]. Dividing cells were isolated from log phase culture and fixed for electron microscopy[3]. Fixation was done in 2.5% gluataraldehyde buffered with 0.1 M sodium caccodylate, pH 7.2 and post-fixed in 1% osmium tetraoxide prepared in the same buffer. Cells were then dehydrated in ethanol series and embedded in Epon-Araldite. Sections were cut with an LKB IV ultramicrotome and stained with uranyl acetate and lead citrate. Sections were observed using an electron microscope (Philips EM 400).

As described earlier[3], the cytospindle microtubules are readily seen in cross-sections of dividing cells (figure 1). Both electron microscopic and immunofluorescent studies have shown that the number and arrangement of microtubules are chronologically linked to the course of fission. From longitudinal sections of dividing cells, it appears that microtubules arise from the lower cytoplasm and enter the longitudinal ridges (figure 2). Observations of kinetosomal region reveal that 2–4, microtubules arise from each kinetosome (figure 3). Cross-sections near the base of kinetosomes reveal microtubules extending to the ridge (figure 4). The fact that permanent microtubule ribbons are also seen in the same region suggests that these microtubules belong to the cytospindle.

Observations from a number of micrographs show that the cytospindle microtubules possibly arise from each kinetosome and enter the longitudinal ridge just like the kinetodesmal fibres. It appears that at least 2–4 microtubules arise from a single kinetosome and then turn right to extend upward towards the anterior. It is not clear from the present observation how long each microtubule extends; but it is apparent that each one extends at least a few microns to cover the length of a few ciliary units before probably terminating into the side of the longitudinal ridge.

The final ringlet arrangement of about 18 microtubules in each ridge (figure 1) is possibly achieved by taking a spiral course as they extend, by the microtubules arising from the kinetosomes of a single kinety. It also appears that each microtubule is linked to the adjacent ones at this stage (figure 1).

Though up to 5 microtubules are seen arising from a single kinetosome (figure 3) not all of them extend towards the longitudinal direction. It appears that at least one or two microtubules traverse transversely. These microtubules are seen deeper in the cortex in many sections. This snows that two sets of microtubules viz. the cytospindle and the transverse microtubules appear in the cortex of dividing paramecia, both originating from the kinetosomes.

Thus the present study reveals that the cytospindle microtubules originating from the base of kinetosomes form part of the kinety as suggested earlier[4]. This is why in mutant cells, with reversed kinety microtubule, bundles are seen on the wrong side of the kinetosomes[4]. Both transverse and cytospindle microtubules possibly play different roles in the process of binary fission.

16 November 1987

1. Jurand, A. and Selman, G. G., *The anatomy of Paramecium aurelia*, MacMillan, London, 1969.
2. Allen, R. D., *J. Cell Biol.*, 1971, **49**, 1.
3. Sundararaman, V. and Hanson, E. D., *Genet. Res. Camb.*, 1976, **27**, 205.
4. Cohen, J., Adoutte, A., Grandchamp, S., Houdebine, L. M. and Beisson, J., *Biol. Cell*, 1982, **44**, 35.
5. Dustin, P., *Microtubules*, Springer-Verlag, Berlin, 1978.
6. Sonneborn, T. M., *Trans. Am. Microsc. Soc.*, 1975, **94**, 155.
7. Sonneborn, T. M., *Methods Cell Physiol.*, 1970, **4**, 241.

## AFLATOXIN PRODUCTION BY *ASPERGILLUS PARASITICUS* ON SEEDS AND CAKES OF *BRASSICA CULTIVARS* UNDER VARIED MOISTURE AND TEMPERATURE REGIME

NEERAJ SHARMA, LAXMI CHAND and G. K. GARG

*Department of Biochemistry, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India.*

SEEDS and cakes of important oil crops like rape and mustard are susceptible to aflatoxin contamination if stored under normal conditions. The present study examines the role of environmental factors on the production of aflatoxins by *A. parasiticus* growing on rape and mustard seeds and cakes.

Seeds of three varieties of rape and mustard viz. T-15 (*Brassica juncea*, Czern, & Cos, Kranti.), T-59 (*B. juncea* Verna) and Sangam (*B. compestris* var. Toria L.) were obtained from the Agronomy Department of the University. A culture of *A. parasiticus* was obtained from the Plant Pathology Department of this University.

Rape and mustard seeds and cakes were autoclaved in bulk at 1.05 kg/cm$^2$ for 15 min. After

**Table 1** *Effect of temperature and moisture on aflatoxin production by A. parasiticus in seeds and cakes of different varieties of rape and mustard*

| | Afla-toxins | $B_1$ | | | $B_2$ | | | $G_1$ | | | $G_2$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety | % RH | 20% | 30% | 40% | 20% | 30% | 40% | 20% | 30% | 40% | 20% | 30% | 40% |
| T-15 | (°C) | | | | | | | | | | | | |
| Seeds | 15 | 1.42 | 3.11 | 12.68 | 3.25 | 9.97 | 11.50 | 0.56 | 1.92 | 8.79 | 1.79 | 6.25 | 15.71 |
| | 30 | 9.53 | 29.34 | 22.83 | 2.60 | 66.17 | 28.03 | 1.11 | 27.61 | 48.84 | 10.73 | 26.66 | 17.11 |
| | 35 | 0.50 | 4.99 | 1.68 | 0.09 | 5.22 | 17.21 | 0.04 | 1.03 | 3.63 | 0.36 | 3.48 | 7.14 |
| Cakes | 15 | 16.87 | 6.41 | 10.19 | 1.13 | 6.51 | 14.34 | 2.44 | 5.39 | 12.13 | 3.40 | 8.04 | 22.58 |
| | 30 | 6.77 | 39.43 | 39.24 | 1.70 | 39.11 | 38.03 | 2.60 | 63.14 | 61.79 | 0.95 | 30.95 | 20.83 |
| | 35 | 0.71 | 2.30 | 19.40 | N.D. | 19.62 | N.D. | 1.85 | 2.82 | 2.39 | 0.89 | 11.93 | 2.92 |
| Sangam | | | | | | | | | | | | | |
| Seeds | 15 | 9.69 | 26.66 | 30.84 | 2.09 | 10.86 | 7.35 | 1.44 | 9.24 | 8.38 | 1.31 | 62.44 | 9.17 |
| | 30 | 10.37 | 21.88 | 57.58 | 6.95 | 32.07 | 41.70 | 4.51 | 12.86 | 87.29 | 10.71 | 27.38 | 66.78 |
| | 35 | 0.84 | 8.91 | 21.44 | 3.04 | N.D. | 9.78 | 0.23 | 1.08 | 6.01 | 7.15 | N.D. | 23.57 |
| Cakes | 15 | 1.00 | 20.96 | 17.35 | 37.39 | 6.44 | 55.06 | 11.70 | 4.79 | 14.31 | 13.59 | 8.04 | 11.61 |
| | 30 | 16.55 | 66.89 | 77.93 | 0.65 | 17.38 | 23.47 | 2.75 | 30.78 | 57.99 | 4.02 | 15.48 | 34.55 |
| | 35 | 1.55 | 4.40 | 3.30 | 0.30 | 0.35 | 2.48 | 0.62 | 0.51 | 2.46 | 0.71 | 1.07 | 1.43 |
| T-59 | | | | | | | | | | | | | |
| Seeds | 15 | 9.29 | 1.31 | 24.94 | 12.91 | 95.20 | 43.45 | 9.18 | 3.25 | 18.89 | 14.70 | 10.73 | 18.98 |
| | 30 | 8.91 | 8.56 | 76.87 | 27.25 | 13.25 | 119.73 | 13.94 | 8.54 | 32.45 | 36.90 | 20.00 | 24.76 |
| | 35 | 0.14 | 0.51 | 2.88 | 2.39 | 1.69 | 0.04 | 0.43 | 0.10 | 0.84 | 2.08 | 0.18 | 0.62 |
| Cakes | 15 | 15.07 | 16.37 | 21.56 | 3.13 | 1.96 | 20.08 | 3.42 | 2.51 | 9.70 | 7.14 | 3.13 | 33.93 |
| | 30 | 11.21 | 11.25 | 80.24 | 11.41 | 28.08 | 106.17 | 31.95 | 31.45 | 67.44 | 7.49 | 28.93 | 50.88 |
| | 35 | 2.45 | 2.93 | 18.25 | 1.30 | 0.45 | 1.39 | 0.86 | 1.02 | 2.39 | 0.18 | 1.25 | 1.18 |

N.D.–Not detectable; $B_1$, $B_2$, $G_1$, $G_2$–Aflatoxins expressed in mg/100 g seeds or cakes.

cooling the seeds and the cakes were distributed individually at the rate of 10 g each in sterilized plates. The samples were inoculated with 0.5 ml of spore suspension[1] and incubated at 15.30 and 35°C for 10 days at 20, 30 and 40% relative humidity[2]. Aflatoxins extracted[3] were subjected to TLC[4] and HPLC and compared with authentic samples obtained from Sigma Chemical Co., USA. Aflatoxin $B_1$, $B_2$, $G_1$, and $G_2$ could thus be tentatively identified and these were confirmed by comparing emission spectrum in a fluorospectrophotometer. Twentyfive microlitre aliquots of the chloroform extracts were subjected to HPLC using a reverse phase column RP-18 and UV detector fixed at 361 nm, the isomeric point of aflatoxins. The peaks were identified by comparing retention time with those of authentic standard samples. Aflatoxins were quantified by measuring the area under different peaks.

The release of aflatoxins was influenced by both temperature and moisture levels (table 1). A moisture regime of 30–40% RH facilitated production of $B_1$, $B_2$, $G_1$ and $G_2$ aflatoxins on seeds and cakes of three varieties. Higher humidity (40% RH) was optimum for aflatoxin production on Sangam and T-59 seeds whereas higher amounts of $B_1$, $B_2$ and $G_1$, $G_2$ were produced on T-15 seeds at 30% RH.

Influence of moisture, temperature and variety on the production of aflatoxins by *A. parasiticus* on cakes was similar to that observed on seeds.

Thanks are due to Dr B. N. Johri for helpful suggestions.

15 December 1987; Revised 23 February 1988

1. Saucer, D. B. and Burroughs, R., *Phytopathology*, 1980, **70**, 516.
2. Vijay Pratap, Singh, B. P. N. and Narain, M.,

In: *Kinetics of water vapour sorption by various powdered food*, 1980.
3. Thomas, R., *J. Assoc. Off. Anal. Chem.*, 1975, **58**, 500.
4. Reddy, T. V., Viswanathan, L. and Venkitasubramanian, T. A., *Anal. Biochem.*, 1970, **38**, 568.

## ON THE BIOTYPES OF ROOT-KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* IN RICE

B. N. SWAIN* and J. S. PRASAD
*Central Rice Research Institute, Cuttack 753 006, India.*
**Present address: Plant Protection Officer, Government of Orissa, Jaleswar 756 032, India*

In the control of endoparasitic nematodes affecting the root system of economic plants, the role of plant varieties/cultures resistant or tolerant to nematodes is recognized as a key factor. Limited evaluation of rice germplasm against the root-knot nematodes revealed a few examples of resistance[1]. But resistant rices evolved at Cuttack against a *M. graminicola* isolate native to Orissa were susceptible when tested at Jorhat against the local population of this nematode[1-3]. This differential behaviour of isolates leads to the possibility of the occurrence of biotypes in this species. An intimate knowledge of the response of rice to different isolates becomes essential for determining resistance to a spectrum of isolates of the nematode. With this objective in view, four isolates of *M. graminicola* were individually inoculated to four resistant and two susceptible rice varieties.

A set of six rice varieties, viz., MW 10, IR 36, Udaya, Daya (all resistant), Annapurna and Parijat (both susceptible), isolated from earlier screening tests[4], was assembled. Each variety was sown in 120 poly-pots at the rate of one seed/pot for inoculation with each of the four isolates to make three replications, each consisting of 10 pots per isolate of nematode. When the seedlings were 5-day-old, 200 freshly hatched 2nd stage juveniles of *M. graminicola* collected from Cuttack (Orissa), Agartala (Tripura), Pattambi (Kerala) and Jorhat (Assam) were inoculated to each seedling. Thirty-five days after inoculation, the plants were uprooted, cleaned and stained in lactophenol cotton-blue[5] and the number of egg masses was recorded.

The results indicated that inoculation with all four nematode isolates was effective and infection occurred in all rice varieties. The number (mean) of egg masses ranged from 0.87 in Udaya to 10.53 in Annapurna for the Cuttack isolate (table 1). In Parijat, the Cuttack and Pattambi isolates produced significantly higher number of egg masses (9.7 and 8.43 respectively) than the Agartala and Jorhat isolates (4.37 and 4.5 respectively). The resistant varieties showed consistent resistance (< 1.20 egg masses) to all four isolates. The number of eggs per egg mass was 23.5 to 35 in resistant varieties and 145.5 to 180.6 in susceptible varieties. Comparison of means (number of egg masses) of isolates indicated no significant difference between the Jorhat, Pattambi and Agartala (3.08–3.42) isolates but the mean for the Cuttack isolate (4.00) was significantly higher than that for the Jorhat isolate. The four resistant rice varieties had mean egg mass numbers between 0.98 (Udaya) and 1.11 (Daya) and were significantly superior to the susceptible Parijat (6.75) and Annapurna (9.57).

**Table 1** *Production of egg masses by four isolates of M. graminicola in resistant (R) and susceptible (S) varieties of rice*

| Rice variety | Isolate | | | | |
|---|---|---|---|---|---|
| | Cuttack | Jorhat | Agar-tala | Pat-tambi | Mean |
| MW 10 (R) | 0 93 | 1.03 | 1 00 | 1 00 | 0 99 |
| IR 36 (R) | 1.00 | 1 07 | 1.03 | 1.03 | 1 03 |
| Udaya (R) | 0 87 | 1 03 | 1.03 | 1 00 | 0.98 |
| Daya (R) | 0.97 | 1 20 | 1.10 | 1 17 | 1.11 |
| Annapurna (S) | 10 53 | 9 63 | 10.23 | 7 87 | 9.57 |
| Parijat (S) | 9.70 | 4 50 | 4.37 | 8.43 | 6 75 |
| Mean | 4 00 | 3 08 | 3.13 | 3 42 | |
| C. D. at: | | 0.05 | 0 01 | | |
| Variety | | 1 09 | 1.45 | | |
| Isolate | | 0 89 | 1 18 | | |
| Variety × Isolate | | 2.17 | 2.89 | | |

Figures are mean numbers of egg masses.

Based on differences in egg mass production between the Cuttack and Jorhat isolates of *M. graminicola* in six rice varieties which included those resistant to the Cuttack (Hamsa) and Jorhat (Basanti) isolates and those tolerant to the two isolates (TKM-6 and Garem respectively), the occurrence of two biotypes in *M. graminicola* was reported earlier[2,3]. Contrary to the report of Sahu and Chawla[6] on the possibility of occurrence of a new biotype in Agartala, the present studies indicate that rice varieties resistant to the Cuttack isolate of *M.*